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Biochemical and functional effects of prenatal and postnatal ω3 fatty acid deficiency on retina and brain in rhesus monkeys

(linolenic acid/docosahexaenoic acid/essential fatty acids)

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ABSTRACT **Docosahexaenoic acid [22:6ω3; 22:6-(4,7,10,13,16,19)] is the major polyunsaturated fatty acid in the photoreceptor membranes of the retina and in cerebral gray matter. It must be obtained either from the diet or by synthesis from other ω3 fatty acids, chiefly α-linolenic acid (18:3ω3). We tested the effect of dietary ω3 fatty acid deprivation during gestation and postnatal development upon the fatty acid composition of the retina and cerebral cortex and upon visual function. Rhesus monkeys (*Macaca mulatta*) were fed semipurified diets very low in 18:3ω3 throughout pregnancy, and their infants received a similar diet from birth. A control group of females and their infants received a semipurified diet supplying ample 18:3ω3. In near-term fetuses and newborn infants of the deficient group, the 22:6ω3 content of phosphatidylethanolamine was one-half of control values in the retina and one-fourth in cerebral cortex. By 22 months of age, the content of 22:6ω3 in these tissues approximately doubled in control monkeys, but it failed to increase in the deficient group. Low levels of 22:6ω3 in the deficient animals' tissues were accompanied by a compensatory increase in longer-chain ω6 fatty acids, particularly 22:5ω6. Functionally, the deficient animals had subnormal visual acuity at 4–12 weeks of age and prolonged recovery time of the dark-adapted electroretinogram after a saturating flash. Abnormally low levels of 22:6ω3 may produce alterations in the biophysical properties of photoreceptor and neural membranes that may underlie these functional impairments. The results of this study suggest that dietary ω3 fatty acids are essential for normal prenatal and postnatal development of the retina and brain.**

Neither linoleic acid (18:2ω6)‡ nor α-linolenic acid (18:3ω3) can be synthesized by animals. Linoleic acid is the precursor of arachidonic acid (20:4ω6) and other longer-chain ω6 fatty acids, whereas linolenic acid is the precursor of the longer-chain ω3 fatty acids, including docosahexaenoic acid [22:6ω3; 22:6(4,7,10,13,16,19)]. These longer-chain fatty acids are important constituents of tissue lipids. The ω6 and ω3 series of fatty acids are not interconvertible.

Linoleic acid was defined as an essential fatty acid 50 years ago, because its absence from the diet leads to overt symptoms including dermatitis, kidney and liver pathology, impaired growth, and reproductive failure (1, 2). The nutritional importance of linolenic acid and the longer-chain ω3 fatty acids, on the other hand, has remained unclear. A deficiency syndrome produced by dietary deprivation of ω3 fatty acids has been defined for fish (3) but not for mammals.

Docosahexaenoic acid (22:6ω3) is the primary ω3 fatty acid in many tissues. The highest levels of 22:6ω3 are found in the retina and in the cerebral cortex, specifically in the phospholipids of photoreceptor outer segment membranes and of synaptosomal membranes. With six double bonds, 22:6ω3 is unique in its high level of polyunsaturation. Depletion of this fatty acid from membranes might be expected to alter their physical and functional properties. In rats, dietary deprivation of ω3 fatty acids has been associated with a decrease in the amplitude of the electroretinogram and an impairment in the ability to learn a visual discrimination (4, 5).

Holman *et al.* (6) described a clinical case of peripheral neuropathy and blurred vision that they ascribed to ω3 fatty acid deficiency. The patient was a child maintained on long-term total parenteral nutrition with a safflower oil emulsion, very low in linolenic acid, as the only source of fat. The symptoms disappeared after the administration of a soybean oil emulsion rich in linolenic acid.

We recently demonstrated in rhesus monkeys that dietary deprivation of ω3 fatty acids during gestation and infancy produced depletion of ω3 fatty acids from the lipids of plasma and an impairment in the development of visual acuity during the first 3 postnatal months (7). In the present paper, we describe the alterations in fatty acid composition of the retina and brain produced by ω3 fatty acid deprivation at different stages of fetal and postnatal development, and we assess the relative impact of maternal versus postnatal dietary manipulation on the fatty acid composition of neural tissues. We also further document changes in visual function, including visual acuity and recovery time of the electroretinogram.§

METHODS

Adult female rhesus monkeys (*Macaca mulatta*) were fed semipurified diets for at least 2 months before conception and throughout pregnancy. The resulting infants were fed liquid semipurified diets from birth. The composition of control and ω3 fatty acid-deficient diets has been previously described in detail (7). For the control mothers and their infants, the fat source was soybean oil, which contains ample amounts of both linolenic and linoleic acids. For the deficient animals, the sole fat source was safflower oil, which contains very low levels of linolenic acid and high levels of linoleic acid. The resulting high ratio of linoleic to linolenic acid suppresses the conversion of linolenic acid to its longer-chain derivatives and thereby exacerbates the effect of the deficient diet. The fatty acid contents of the experimental diets are presented in Table 1. The fat content for the adult diets was 5% by weight or 13.4% of kcal, and for the infant formula diets it was 15% by weight or 30% of kcal. There were no differences between the two groups in food intake or body weight.

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‡Fatty acid nomenclature: first number indicates length of carbon chain; second number, after colon, specifies number of double bonds; third number, after ω, gives number of carbons before first double bond, counting from the methyl end of the chain.

§Preliminary reports of parts of this work were presented at the Annual Meetings of the Association of American Physicians, Washington, DC, May 1984, and the Association for Research in Vision and Ophthalmology, Sarasota, FL, May 1985, and appeared in refs. 8 and 9.

Table 1. Fatty acid compositions of control and ω3 fatty acid-deficient semipurified diets for rhesus monkeys

Fatty acid	% (wt/wt) of total fatty acids	
	Control diet (soybean oil)	Deficient diet (safflower oil)
Saturated	14.9	9.6
18:1ω9	23.7	13.3
18:2ω6	53.1	76.0
Total ω6	53.4	76.5
18:3ω3	7.7	0.3
22:6ω3	0	0
Total ω3	7.7	0.3
ω6/ω3	7	255

Two procedures were used to assess the functional integrity of the retina and visual system. First, the visual acuity of the infants was measured behaviorally at 4, 8, and 12 weeks of age by the preferential looking method, as described in a previous paper (7). Briefly, this method takes advantage of human or animal infants' strong inherent preference for looking at patterned stimuli as a means to determine their ability to detect black and white high-contrast stripes within an unpatterned gray background. Responses to stripes of different widths are used to define a threshold for visual acuity. Second, the electroretinogram, a potential change evoked from the retina by light stimulation, was recorded with a contact lens corneal electrode while the animals were under thiamylal anesthesia. Recording and stimulating procedures have been described previously (10). For the purposes of this paper, particular attention was given to the response of the dark-adapted eye to saturating flashes, that is, those sufficiently bright to evoke a response of maximal amplitude [intensity = 0.5 millilambert·sec (1.6 cd/m^2·sec)]. Flashes of this intensity must be presented at intervals of 20 sec or more to allow for full recovery of the response between flashes. In this study, these flashes were presented at intervals varying from 3.2 to 30 sec, to determine the degree to which the response was reduced by the shorter intervals and the time required for the response to recover to full amplitude.

At approximately 22 months of age, 2 animals in each diet group were killed while they were under deep ketamine anesthesia, and tissues, including occipital and frontal cerebral cortex (gray matter) and retina, were taken for lipid fatty acid analyses. In 2 additional 22-month-old deficient animals, 20- to 30-mg biopsy samples of frontal cortex were obtained by craniotomy. Tissues were also taken from 10 fetuses and infants, 5 from each diet group, that were born prematurely, were stillborn, or died of causes unrelated to the experimental treatment. The ages of these fetuses and infants in the deficient group were 130 and 132 days postconception (average full-term gestation for rhesus monkeys = 165 days) and 0, 6, and 16 days after birth. For the control group, the ages were 160 days postconception and 11, 12, 16, and 18 days after birth. Tissues were obtained within 2 hr of death and were stored at −20°C until analyzed.

Tissue lipids were extracted by the method of Folch *et al.* (11), and thin-layer chromatography was utilized to separate the phospholipid classes of occipital and frontal cerebral cortex (12) and retina (13). The fatty acids of each phospholipid class were methylated and were then analyzed by capillary column gas–liquid chromatography (14).

Differences in the content of selected fatty acids as a function of diet and age were evaluated by 2 × 2 factorial analysis of variance and, when overall F values indicated significant differences, by calculation of the F value for simple effects of diet within each age group and simple effects of age within each diet group (15).

RESULTS

At all ages, animals of the ω3 fatty acid-deficient group had considerably lower levels of ω3 fatty acids in tissue phospholipids than their controls. Levels of the major fatty acids in phosphatidylethanolamine—the most prominent phospholipid containing high levels of polyunsaturated fatty acids—are presented in Table 2 for the occipital cortex, frontal cortex, and retina. Fig. 1 depicts graphically the data for occipital cortex. In near-term fetuses and infants of the deficient group, the phosphatidylethanolamine of occipital cortex contained one-fourth as much 22:6ω3 as in control newborns (Fig. 1*A*). However, in deficient tissues there was a notable compensatory increase in the longer-chain ω6 fatty

Table 2. Major fatty acids of brain and retinal phosphatidylethanolamine in control and ω3 fatty acid-deficient rhesus monkeys at

Fatty acid*	Occipital cortex				Frontal cortex			
	Control		Deficient		Control		Deficient	
	Perinatal (n = 5)	22 months (n = 2)	Perinatal (n = 5)	22 months (n = 2)	Perinatal (n = 5)	22 months (n = 2)	Perinatal (n = 5)	22 months (n = 4)
18:0	30.1 ± 2.9	30.5 ± 0.1	24.9 ± 5.7	26.9 ± 5.9	24.1 ± 3.2	31.4 ± 2.2†	26.7 ± 2.5	29.6 ± 2.9
Total saturated	38.5 ± 2.7	38.2 ± 2.5	36.7 ± 4.5	37.4 ± 5.4	30.7 ± 3.4	39.2 ± 1.6†	38.8 ± 4.1¶	39.2 ± 2.2
18:1ω9	5.9 ± 1.2	7.9 ± 0.3	7.4 ± 1.3	7.0 ± 0.1	6.9 ± 0.8	7.1 ± 1.2	8.5 ± 1.8	6.9 ± 0.6
Total mono	10.2 ± 3.1	10.8 ± 2.2	15.0 ± 1.8	9.1 ± 0.8	12.2 ± 2.9	10.3 ± 1.6	13.9 ± 3.9	10.1 ± 1.3
20:4ω6	16.8 ± 2.0	7.9 ± 0.4‡	18.0 ± 4.4	10.2 ± 0.9	17.9 ± 2.1	12.4 ± 0.9†	16.2 ± 1.7	12.6 ± 1.5†
22:4ω6	12.9 ± 4.0	5.3 ± 0.2‡	9.8 ± 1.6	10.2 ± 0.1¶	11.8 ± 1.7	9.4 ± 0.8	10.4 ± 2.2	11.6 ± 2.0
22:5ω6	3.7 ± 0.2	0.5 ± 0.1‡	10.5 ± 2.4¶	22.5 ± 2.3‡¶	4.0 ± 1.2	1.4 ± 0.3†	11.0 ± 3.2¶	18.3 ± 2.5‡¶
Total ω6	33.7 ± 3.6	15.7 ± 0.2‡	40.7 ± 5.6§	45.3 ± 3.3¶	35.8 ± 4.3	24.9 ± 1.5†	39.9 ± 6.2	44.2 ± 4.0¶
22:6ω3	15.0 ± 2.5	34.0 ± 0.8‡	4.0 ± 2.3¶	5.8 ± 0.6¶	15.6 ± 3.2	22.3 ± 0.3†	3.5 ± 1.6¶	3.8 ± 0.4¶
Total ω3	15.2 ± 2.3	35.0 ± 0.7‡	4.0 ± 2.3¶	6.0 ± 0.5¶	16.3 ± 3.5	22.5 ± 0.5	3.5 ± 1.6¶	3.8 ± 0.4¶
Total ω6 + ω3	48.9 ± 5.4	50.7 ± 0.5	44.7 ± 6.0	51.4 ± 2.9	52.1 ± 6.5	47.3 ± 1.0	43.4 ± 6.6	48.0 ± 3.7

All results are percent (wt/wt) of total fatty acids, presented as mean ± SD.

*Total saturated includes all saturated fatty acids with 12 to 24 carbons. Total mono includes the following monounsaturated fatty acids: 14:1, 24:1ω9. Total ω6 includes *cis*-18:2ω6, 18:3ω6, 20:2ω6, 20:3ω6, 20:4ω6, 22:4ω6, and 22:5ω6. Total ω3 includes *cis*-18:3ω3, 18:4ω3, 20:3ω3, 20:4ω3,

†‡Significant difference between ages within each diet group: †, $P < 0.05$; ‡, $P < 0.01$ (two-tailed).

§¶Significant difference between diet groups at a given age: §, $P < 0.05$; ¶, $P < 0.01$ (two-tailed).

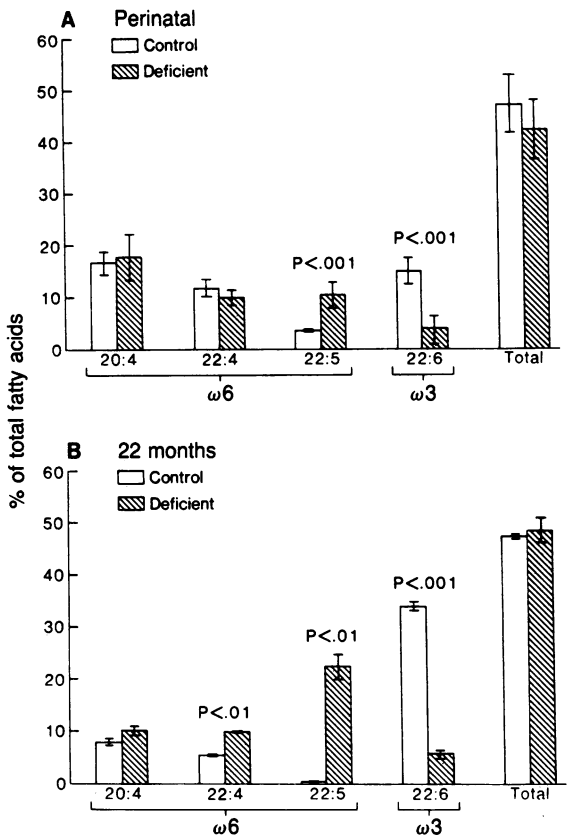

FIG. 1. Major ω6 and ω3 fatty acids of phosphatidylethanolamine in the occipital cortex of rhesus monkeys during the perinatal period (*A*) and at 22 months of age (*B*): Comparison of control (open bars) and ω3 fatty acid-deficient (hatched bars) groups. Values are expressed as percent (wt/wt) of total fatty acids (mean ± SD). *P* values are given for significant differences between diet groups at each age. "Total" is the sum of the four individual fatty acids for which data are shown.

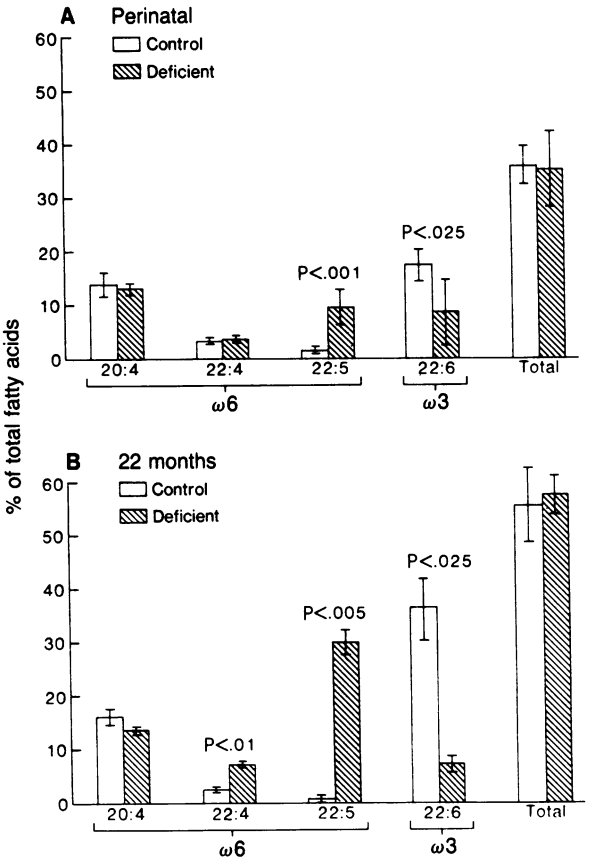

FIG. 2. Major ω6 and ω3 fatty acids of phosphatidylethanolamine in the retina of rhesus monkeys during the perinatal period (*A*) and at 22 months of age (*B*). See legend for Fig. 1.

acid, 22:5. Therefore, despite large alterations in the amount of 22:6ω3, the sum of 20- and 22-carbon polyunsaturated fatty acids (ω3 plus ω6) remained essentially unchanged. The same pattern was seen in frontal cortex (Table 2). As regards the retina (Fig. 2*A*), the perinatal deficient group had half as much 22:6ω3 in phosphatidylethanolamine as control newborns, and they showed the same compensatory increase in 22:5.

Between birth and 22 months of age, the percentage of 22:6ω3 in the phosphatidylethanolamine of the occipital cortex more than doubled in control animals (Fig. 3*A*). In deficient animals, on the other hand, concentrations of 22:6ω3 failed to increase (Fig. 3*B*) and at 22 months they were only one-sixth of control values (Fig. 1*B*). The ω6 fatty acid 22:5 declined from its already low perinatal levels in controls, whereas in the deficient animals, the proportion of this fatty acid doubled. This ω6 fatty acid reached levels 45 times greater than in controls, and thereby almost completely replaced 22:6ω3. Another long-chain ω6 fatty acid, 22:4, was also increased in the deficient group. Thus, as the deficient animals matured, the total 20- and 22-carbon polyunsaturated fatty acids continued to be maintained at control levels despite low levels of 22:6ω3. The same pattern of developmental changes occurred in the retina (Fig. 2*B*) and in frontal cortex (Table 2) between the perinatal period and 22 months of age.

Similar patterns were found in other phospholipid classes. Phosphatidylserine was most similar to phosphatidylethanolamine in that it normally contained high levels of polyunsaturated fatty acids, including 22:6ω3; it was similarly affected by age and by ω3 fatty acid deficiency. Phosphatidylcholine and phosphatidylinositol, although they contained far less 22:6ω3 than the other two classes, also had similar percent reductions of 22:6ω3 content in the tissues of deficient animals.

different ages

Retina			
Control		Deficient	
Perinatal (n = 5)	22 months (n = 2)	Perinatal (n = 4)	22 months (n = 2)
29.6 ± 6.6	23.8 ± 6.0	30.5 ± 1.9	28.6 ± 1.0
42.8 ± 5.6	30.6 ± 4.9†	45.0 ± 8.0	32.7 ± 2.6
7.9 ± 1.4	5.9 ± 1.1	8.9 ± 1.6	4.2 ± 0.4†
11.2 ± 1.1	8.8 ± 1.1	12.2 ± 3.3	5.0 ± 0.3†
13.9 ± 2.3	16.0 ± 1.6	13.2 ± 1.2	13.4 ± 0.7
3.3 ± 0.6	2.4 ± 0.4	3.7 ± 0.8	7.2 ± 0.4‡¶
1.5 ± 0.6	0.7 ± 0.7	9.6 ± 3.3¶	29.8 ± 2.4‡¶
22.6 ± 3.0	22.6 ± 2.3	29.8 ± 4.3§	57.7 ± 3.0‡¶
17.3 ± 2.9	36.4 ± 5.8‡	8.6 ± 6.0§	7.1 ± 1.6§
18.7 ± 3.2	37.2 ± 4.7‡	8.9 ± 6.2§	7.3 ± 1.6§
41.2 ± 6.0	59.8 ± 7.0†	38.7 ± 8.0	65.0 ± 1.4†

16:1ω7, 17:1ω9, *trans*-18:1, 18:1ω9, 20:1ω9, 22:1ω11, 22:1ω9, and 20:5ω3, 22:5ω3, and 22:6ω3.

These alterations in retina and brain fatty acid composition of the deficient animals were associated with two functional abnormalities. First, electroretinographic recordings, performed at 21 months of age, or within 1 month of the biochemical analyses, demonstrated an impairment in the recovery of the dark-adapted response to saturating flashes—i.e., those flashes sufficiently bright to elicit maximal response amplitudes. At long intervals (20 sec or more), responses were as large in deficient animals as in controls. However, the suppressive effect of shorter intervals was much greater in deficient animals (Fig. 4). At 3.2-sec intervals, responses were reduced to 74% of maximal amplitude in control animals; in deficient animals, they were reduced far more, to 51% of maximal amplitude. Fig. 4 includes individual values from the same animals from which tissues were taken at 22 months for fatty acid analyses. The mean time required for recovery of the response to 80% of its full amplitude was 3.9 sec (SEM = 0.3 sec) in the control group and 6.2 sec (SEM = 0.4 sec) in the deficient group.

The second functional deficit was demonstrated by behavioral tests of visual acuity. Deficient infants had significantly higher visual acuity thresholds (that is, poorer acuity) at 4–12 weeks of age (Fig. 5). These data, some of which were reported previously (7), now include results for six new animals. Highlighted in the figure are individual values for the animals for which biochemical results are described above. Thus, ω3 fatty acid deficiency was accompanied by a visual sensory deficit as well as an impairment in the physiological response of the retina.

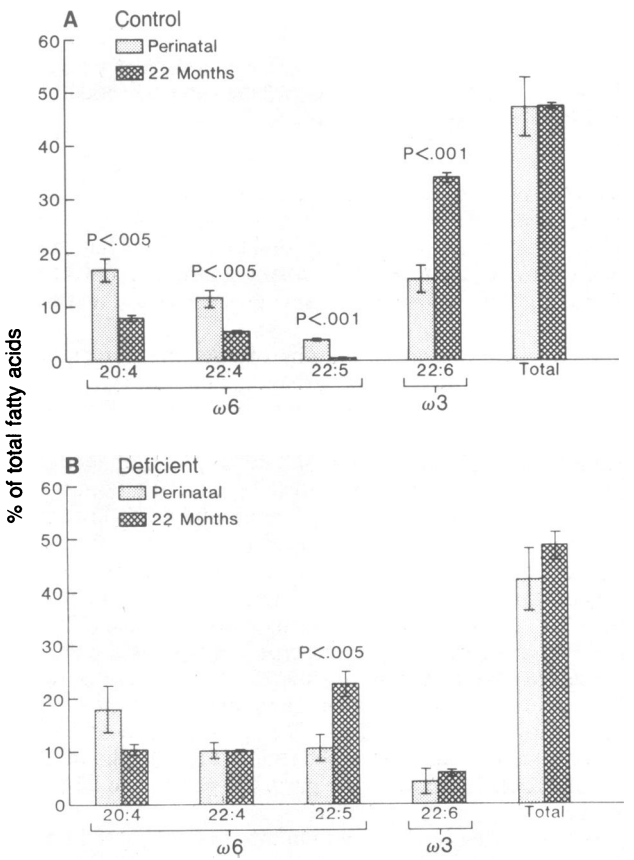

FIG. 3. Major ω6 and ω3 fatty acids of phosphatidylethanolamine in the occipital cortex of control (*A*) and ω3 fatty acid-deficient (*B*) rhesus monkeys: Comparison of perinatal (stippled bars) and 22-month-old (cross-hatched bars) groups. Values are expressed as percent (wt/wt) of total fatty acids (mean ± SD). *P* values are given for significant differences between ages within each diet group. "Total" is the sum of the four individual fatty acids for which data are shown.

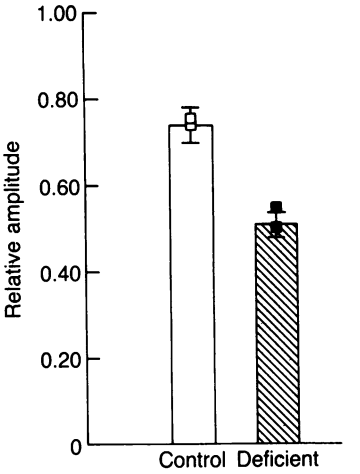

FIG. 4. The effect of short (3.2-sec) intervals between flashes on the amplitude of the dark-adapted electroretinogram B-wave response. Relative amplitude (mean ± SEM) represents response amplitude at 3.2-sec intervals as a fraction of the maximal response amplitude obtained at intervals of 20 sec or more. Maximal amplitudes did not differ between groups, but there was a significant difference ($P < 0.01$ by Student's *t* test) in relative amplitude—i.e., the responses of deficient animals were more affected by reducing the interval between flashes. Squares indicate values for those individual animals for which retinal and brain fatty acid composition was determined at 22 months. For control group, $n = 7$; for deficient group, $n = 6$.

DISCUSSION

These results demonstrate that the high content of ω3 fatty acids normally present in the cerebral cortex and retina is greatly reduced by a combination of maternal and postnatal dietary deprivation in rhesus monkeys. Most importantly, this depletion is associated with functional changes in the retina and visual system. Similar biochemical results were previously reported in rats for whole brain (5, 16) and retina (16) after two generations of ω3 fatty acid deprivation.

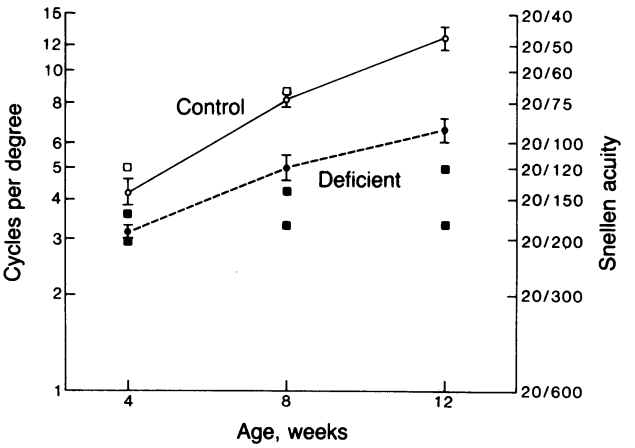

FIG. 5. Visual acuity thresholds (mean ± SEM) as determined by the preferential looking method for control and ω3 fatty acid-deficient infant monkeys. Thresholds are expressed in cycles per degree of visual angle and in the equivalent Snellen values. Mean values for the control group (○) include measurements from 8 infants at 4 and 8 weeks and 6 at 12 weeks; mean values for the deficient group (●) include values for 10 subjects at 4 weeks, 11 at 8 weeks, and 12 at 12 weeks. Differences between groups were statistically significant at each age as determined by Student's *t* test ($P < 0.01$ at 4 weeks, $P < 0.001$ at 8 and 12 weeks). Squares (□, control; ■, deficient) represent individual values for those animals for which biochemical data are presented. Acuity measurements were not obtained for one such control monkey.

However, rats are poor models of human nutritional requirements because they have large litters, grow rapidly, and are born at a much more immature stage of development than primate infants (17). Rats are particularly different from higher primates in retinal structure and visual function, whereas rhesus monkeys closely resemble humans in both of these respects.

We previously reported that ω3 fatty acids were depleted slowly from the plasma lipid classes of pregnant female monkeys during dietary ω3 fatty deprivation and that plasma levels of docosahexaenoic acid (22:6ω3), although reduced in the deficient neonates, were higher than in their mothers (7). These findings suggested that the developing fetus might be preferentially supplied with 22:6ω3 via the placenta and thereby protected from the effects of maternal dietary ω3 fatty acid deprivation. However, the abnormally low levels of 22:6ω3 in the tissues of near-term fetuses and newborn infants of deficient mothers demonstrated that maternal dietary deprivation of ω3 fatty acids has a significant impact upon fetal tissues.

Vulnerability to dietary ω3 fatty acid deprivation appeared to be even greater postnatally. The rapid decline of plasma phospholipid levels of 22:6ω3 after birth in deprived infants (7) suggested enhanced susceptibility to ω3 fatty acid deficiency once the infants were removed from maternal sources. Indeed, the cerebral cortex and retina of these monkeys failed to increase their percentage content of 22:6ω3 postnatally. In contrast, the tissues of control animals doubled their proportion of 22:6ω3 between birth and 22 months of age. The normal postnatal accretion of 22:6ω3 differs from the developmental pattern for DNA, an index of cell number, which achieves adult levels in rhesus monkey brain by the time of birth (18). Thus, the normal postnatal increase in 22:6ω3 may reflect processes involved in cell growth and differentiation, such as the proliferation of synaptic connections.

The fatty acid compositions of the cerebral cortex described here for control newborn and juvenile rhesus monkeys are very similar to those reported by Svennerholm (19) for human newborns and adolescents, respectively. However, the brain and retina of human infants are less developed at birth than those of rhesus monkeys (17), so that human infants might be even more vulnerable to postnatal dietary deprivation of ω3 fatty acids.

We have now demonstrated that depletion of 22:6ω3 from the retina is associated with delayed recovery of the dark-adapted electroretinogram and with an impairment in the development of visual acuity. The effect on visual acuity could also be related to ω3 fatty acid depletion of occipital cortex, which is the primary visual cortex, and other parts of the central visual system. Reduced levels of 22:6ω3 may alter the structure and function of retinal photoreceptors and/or the central visual system by affecting membrane fluidity and permeability, by altering the activity of membrane-bound enzymes or transport systems, or by other mechanisms that are not yet understood. Our findings provide evidence that dietary ω3 fatty acids are essential for normal prenatal and postnatal development of the retina and brain. Further research will be required to determine the relative contributions of prenatal versus postnatal deprivation to the observed functional deficits and to determine the degree to which the biochemical and functional effects of ω3 fatty acid deficiency are reversible.

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